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Isolation of Varicella-Zoster Virus From Vesicles in Children With Varicella

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Varicella-zoster virus (VZV) was isolated from 29 samples of the vesicular fluid in 13 otherwise healthy children with varicella who were aged from 7 months to 7 years. Human embryonic *lung cells* were used for viral isolation, and VZV was identified by a characteristic cytopathic effect and an indirect immunofluorescence assay. VZV was found in 17 samples; in two (12%) of which it was also detected after filtration (0.45 μm). The rate of isolation was 100% in the first two days after the onset of the disease. It declined gradually with time to 1 of 6 in the samples 6 days after the clinical onset. Specific IgG antibody to VZV was investigated in the same materials. The positive rate was 0% (0/13) in the first 3 days and increased to 7 of 16 in the following 3 days after the onset. VZV was not isolated from samples with specific antibody. In conclusion, VZV can be isolated easily from vesicles within the first 3 days of onset, but the filtration of samples affects its isolation. Infective VZV disappears gradually in vesicles after the first 3 days, and this may be related to the establishment of immune reactions including specific antibody.

KEY WORDS: varicella-zoster virus, viral isolation, vesicle

INTRODUCTION

Varicella is a common and highly contagious disease of childhood. As the first event of varicella-zoster virus (VZV) infection in a susceptible person, Grose [1981] postulated that the virus would gain entry through the respiratory mucosa and presumably multiply in the regional lymphatic tissue. The application of the polymerase chain reaction (PCR) detected VZV DNA in throat swabs or nasopharyngeal secretions of exposed and susceptible individuals [Ozaki et al., 1991; Connelly et al., 1993], thus supporting this hypothesis. VZV is assumed to spread from a patient by droplet nuclei or air droplets, because airborne transmission of varicella in hospitals has been demonstrated [Leclair et al., 1980; Gustafson et al., 1982].

VZV can be isolated readily from the vesicular fluid obtained within the first 3 days of the rash, although it is difficult to isolate VZV from pharyngeal swabs [Trlifajova et al., 1984; Ozaki et al., 1989]. In the present study, VZV was isolated from vesicles of otherwise healthy children with varicella in relation to the time after the clinical onset. Moreover, the specific antibody was investigated in the vesicular fluid and the sera of the patients.

MATERIALS AND METHODS

Study Population

This study was conducted at the pediatric outpatient clinic of Showa Hospital over an 8-month period in 1994. Thirteen otherwise healthy children with varicella diagnosed by characteristic skin lesions of primary VZV infection were included in this study with an age range from 7 months to 7 years. All had typical varicella, received no antiviral treatment, and recovered completely. Informed consent was obtained from the parents.

Preparation of Clinical Samples

For virus isolation and antibody detection, an attempt was made to take vesicular fluid from the patients serially 1 to 3 times after the appearance of rash. A very small amount of fluid was drawn into a syringe from 6 vesicles, and placed in 1.0 ml of the sampling medium (phosphate-buffered saline with 5% sucrose, 0.1% sodium glutamate and 10% fetal calf serum). Samples could not be obtained later than 6 days after the clinical onset, because the lesions became crusted. A total of 29 samples were taken and stored at −80°C before analysis. Paired sera were also obtained from every patient and measured for antibody to VZV.

Viral Isolation From Vesicles

Human embryonic lung (HEL) cells were used for virus isolation; 0.4 ml of every sample was inoculated directly onto the cell cultures, and at the same time, another 0.4 ml was inoculated after filtration through a 0.45 μm filter. The cultures were observed daily for one week, and even if no cytopathic effect (CPE) was

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TABLE I. Serum Antibody to VZV

Patient No.	Age/Sex	IgG antibody[a] (day[b] of the disease)
1	7Y/M	7.8(1), 47.3(8)
2	7M/F	2.3(1), 13.0(8)
3	1Y/F	<2.0(1), 6.9(5)
4	3Y/M	3.5(1), 39.4(8)
5	4Y/M	<2.0(2), 22.2(8)
6	10M/F	<2.0(1), 16.8(10)
7	3Y/M	13.6(1), 62.3(6)
8	1Y/F	<2.0(1), 12.0(8)
9	5Y/F	<2.0(1), 10.6(7)
10	3Y/F	<2.0(2), 8.2(7)
11	5Y/F	<2.0(1), 12.0(8)
12	5Y/M	<2.0(0), 6.2(4)
13	1Y/F	<2.0(1), 14.0(8)

[a]By ELISA: cut-off index, 2.0.
[b]Day 0 is the day of appearance of rash.

TABLE II. Viral Isolation and Antibody in Vesicles

Patient No.	Day 0[a]	1	2	3	4	5
	Isolation positive (antibody[b] positive)					
1				−(+)		−(+)
2		+[c](−)				+(−)
3		+(−)		−(−)		−(+)
4		+(−)		−(+)		−(+)
5			+(−)			
6				+(−)		
7		+(−)		−(+)		
8		+(−)		+(−)		−(−)
9		+(−)	+[d](−)			−(+)
10			+(−)		+(−)	
11		+(−)		−(−)		
12	+[c](−)		−(−)		−(−)	
13		+(−)		+(−)		
Positive rates	1/1 (100%)	8/8 (100%)	3/4 (75%)	3/8 (37.5%)	1/2 (50%)	1/6 (16.7%)

[a]Day 0 is the day of appearance of rash.
[b]By indirect immunofluorescence assay (IgG).
[c]VZV was isolated from the filtrated sample as well as from the unfiltrated one.
[d]CPE observed after a blind passage.

observed, the cell suspension treated with trypsin was mixed with a newly prepared HEL cell suspension in a growth medium. Observation continued for a further period of about 2 weeks. When no CPE was detected at the end of the observation period, the result was considered to be negative. Viral isolate was identified first by a characteristic CPE for VZV. An indirect immunofluorescence assay with a monoclonal antibody to VZV was then used for confirmation.

Antibody to VZV

Detection of VZV antibody in vesicular fluid was carried out by an indirect immunofluorescence assay. HEL cells infected with VZV were fixed on a slide with acetone. Ten μl of the sample was placed on the slide, and incubated at 37°C. After incubation for 30 minutes, the slide was washed with phosphate-buffered saline and 1 drop of fluorescein-conjugated rabbit antibody to human IgG was added, and the slide was incubated for another 30 minutes at 37°C. After washing with phosphate-buffered saline, the cells on the slide were examined under a fluorescent microscope.

Paired sera obtained from the patients were sent to Special Reference Laboratories Inc. (Tokyo, Japan) for measuring IgG antibody responses to VZV by enzyme-linked immunosorbent assay (ELISA) carried out in blood samples diluted at 1:200.

RESULTS

The age, sex, and serum antibodies to VZV of the 13 varicella patients are shown in Table I. Seroconversion was observed in 9 patients. In 4 of 10 patients, specific IgG antibodies were detected on the day after the appearance of rash.

Table II shows the results of viral isolation and antibody detection in 29 vesicular samples during 6 days of the disease. The volume of those vesicular samples was very small and was not measured. VZV was found in 17 samples, in only 2 of which it could be also found after filtration. The rate of the isolation was 100% during the first 2 days after the onset of the disease. It declined gradually with time and was 17% (1/6) in the samples of the 6th day of the disease. Except for one material, no additional blind passage was needed for virus isolation.

Specific IgG antibody to VZV was detected in 7 of 29 vesicular samples. Antibody was not detected (0/13) during the first 3 days and increased to 44% (7/16) in the following 3 days after the onset. VZV was not isolated from vesicular fluid with detection of specific antibody.

DISCUSSION

Although it is known that VZV can be isolated readily from vesicular fluid of varicella patients during the early phase, the frequency of isolation has not been demonstrated accurately. VZV DNA was detected by PCR in almost all samples of crusts as well as vesicular samples [Kido et al., 1991]. In contract with the results by PCR, the isolation rate by tissue culture which was 100% during the first 2 days declined gradually with time after the clinical onset. In other words, if varicella patients spread VZV only from their skin lesions, they would become noncontagious even when the vesicles are still present.

In a previous study, VZV could not be isolated from pharyngeal swabs of children with varicella after filtration [Ozaki et al., 1989]. In the present study the isolation rate was also affected and decreased to 12% (2/17) by filtration of samples. Polymorphonuclear leukocytes, macrophages, lymphocytes and epithelial cells are thought to be present in vesicular fluid in varicella as in the case in zoster [Stevens et al., 1975]. A potential explanation for the effect of filtration is that infective VZV is more likely to be present in cell-associated form in vesicles than in cell-free form.

Antibody in the vesicular fluid became detectable on the 4th day of the disease, 2 days later than its appearance in serum. As far as we know, this is the first study investigating antibodies in vesicles of varicella. VZV or

VZV-infected cells might be inactivated by the specific immunities established in vesicles because VZV was not isolated from the vesicular samples with detection of specific IgG antibody to VZV. But the other specific antibodies (e.g., IgM and IgA) and the cellular immunity which is also considered to be important in inactivating VZV or VZV-infected cells were not investigated in this trial.

In the present study it was demonstrated that the frequency of VZV isolation from vesicles of otherwise healthy children with varicella was 100% during the first 2 days and decreased to 17% gradually with time after the appearance of the rash. A varicella patient might be noncontagious even at the time the vesicles are still present.

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